

APPENDIX

Polyamides Effects on HTLV-I Transcription

The text of the appendix was taken in part from a manuscript co-authored with Jill A. Livengood, Professor Jennifer K. Nyborg (Colorado State), and Professor Peter B. Dervan (Caltech).

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Abstract

Human T-cell leukemia virus type-1 (HTLV-1) depends on the virally encoded transcription factor Tax for efficient viral replication and gene expression. In a complex with CREB, Tax contacts the minor groove of the promoter DNA at guanine and cytosine rich sequences that flank three of the consensus cyclic-AMP response elements (CREs). In this study, we used six Tax-directed pyrrole-imidazole polyamides specifically designed to block Tax binding to DNA at each GC sequence of the three viral CREs. We found that four of these polyamides disrupt binding of the Tax/CREB complex *in vitro* and that these same molecules also inhibit Tax-mediated transcription *in vitro* on chromatin-assembled templates. However, of these four Tax/CREB-specific polyamides, only one polyamide appears to be uniquely Tax specific. We show that polyamides can enter the nuclei of HTLV-1 infected T-cells, and two of the four polyamides down-regulated virion production in these cells. Together, these data illustrate the importance of studying polyamide inhibition of gene expression *in vitro* and *in vivo*, as the function of the polyamides in living cells is not fully understood. Finally, our data indicates that targeted disruption of the Tax/CREB complex, or other complexes which assemble on the HTLV-1 promoter, may provide a novel approach for inhibiting viral replication *in vivo*.

Introduction

Human T-cell leukemia virus type-1 (HTLV-1) is a retrovirus responsible for an aggressive and fatal malignancy called adult T-cell leukemia (ATL)^{1,2}, and a neurodegenerative disorder called tropical spastic paraparesis (TSP/HAM).³⁻⁶ In an infected T-cell, the virus is believed to remain latent until an ill-defined signal triggers expression of the virally-encoded transcription factor called Tax. A prominent role for the transcription factor Tax has been established in the etiology of both ATL and TSP/HAM. Perhaps integral to these effects on pathogenesis, Tax has also been shown to promote high-level virion production and to accelerate cell division of the HTLV-I-infected cell.⁷

To stimulate transcription, Tax binds to three imperfectly conserved 21 base-pair repeats in the HTLV-1 promoter called viral CREs (cyclic AMP response elements).⁶ The viral CREs are composed of an off-consensus cAMP response element immediately flanked by GC-rich sequences. Both the CRE core and the GC flanking sequences are essential for efficient transcription. Tax makes protein-protein contacts with the transcription factor CREB while also making protein-DNA contacts with the minor groove of the GC rich sequences of the viral CRE.⁸⁻¹⁴ The Tax-CREB complex then recruits the cellular co-activator CBP/p300 to the promoter.¹⁵⁻¹⁸ The ternary complex containing Tax, CREB, and CBP/p300 is essential for high levels of HTLV-I transcription.^{10, 12-20}

Polyamides are small molecules containing imidazole (Im) and pyrrole (Py) heterocyclic amino acids that bind in the minor groove of DNA.^{21, 22} They can be designed to bind to the DNA with very high sequence specificity and have been shown to inhibit transcription factor binding to a variety of cis-acting elements.²³ Previously, we

have used polyamides to target the minor groove GC-rich sequence of the third viral CRE of HTLV-I.²⁴ This study showed that designed polyamides specifically displaced Tax from the viral CRE DNA, providing additional strong evidence that Tax binds DNA specifically at these sequences.²⁴ Furthermore, at concentrations where the polyamides specifically blocked the Tax-DNA interaction, they also inhibited Tax transactivation *in vitro* from a promoter carrying reiterated copies of the third viral CRE. This study was limited in scope, as the polyamides bound their target sequences with low affinities, and only the third viral CRE was targeted by polyamides. Sequence variation among the three viral CREs prohibited inhibition of Tax binding at all three sites.

In this study, six hairpin polyamides (**U1**, **D1**, **U2**, **D2**, **U3**, and **D3**) were designed to target an upstream (U) or a downstream (D) Tax binding site at each of the three viral CREs within the HTLV-1 promoter. In addition, a seventh polyamide (**C**) was synthesized as a control that bound the HTLV-1 promoter but at sequences between the viral CREs. We hypothesized that these polyamides would block the binding of Tax at each of the individual viral CREs simultaneously, thus serving as inhibitors of Tax function on the natural HTLV-I promoter both *in vitro* and *in vivo*. If these molecules are effective at disruption of Tax binding, they may inhibit replication of the virus *in vitro* and, by extension, in cell culture experiments.

Of the hairpin polyamides **U1**, **D1**, **U2**, **D2**, **U3**, and **D3**, three of these molecules reduced binding of the Tax/CREB complex, and only one demonstrated specific inhibition of Tax transactivation *in vitro*. We show that polyamides readily enter the nucleus of living HTLV-1-infected T-cells and that two of the polyamides downregulated HTLV-1 virion production in living cells. However, the two polyamides that inhibited

viral replication in cell culture had no Tax-specific effects *in vitro*, suggesting that the mechanisms of polyamide inhibition are not clearly understood and emphasizing the importance of investigating polyamide function in living cells. Finally, our data suggest that further development of sequence-specific DNA binding polyamides may provide a novel approach to disrupting viral replication.

Results

Design and Synthesis of Polyamides

The HTLV-1 promoter consists of three imperfectly conserved 21 bp repeats referred to as viral CRE 1, 2, and 3 (Figure A.1). These sequences are highly similar in nucleotide sequence but non-identical. Expression of HTLV-1 via the viral CREs is dependent upon the virally-encoded Tax protein, and all three viral CREs have been shown to contribute to Tax-activated transcription.³⁵⁻³⁷ To further investigate a role for polyamides in the inhibition of Tax-mediated viral transcription, we targeted each of the three viral CREs with specific, rationally designed polyamides (Figure A.2). Polyamides were designed to bind both of the 5' and 3' GC-rich regions on the three viral CREs where Tax makes DNA contacts in the minor groove.^{8,9,24} The polyamides were named

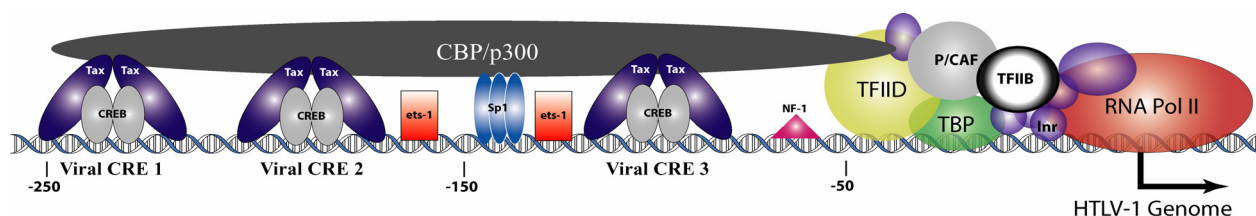


Figure A.1 Schematic of the HTLV-1 promoter showing the preinitiation complex at the promoter start site and the Tax/CREB binding sites of the three viral CREs.

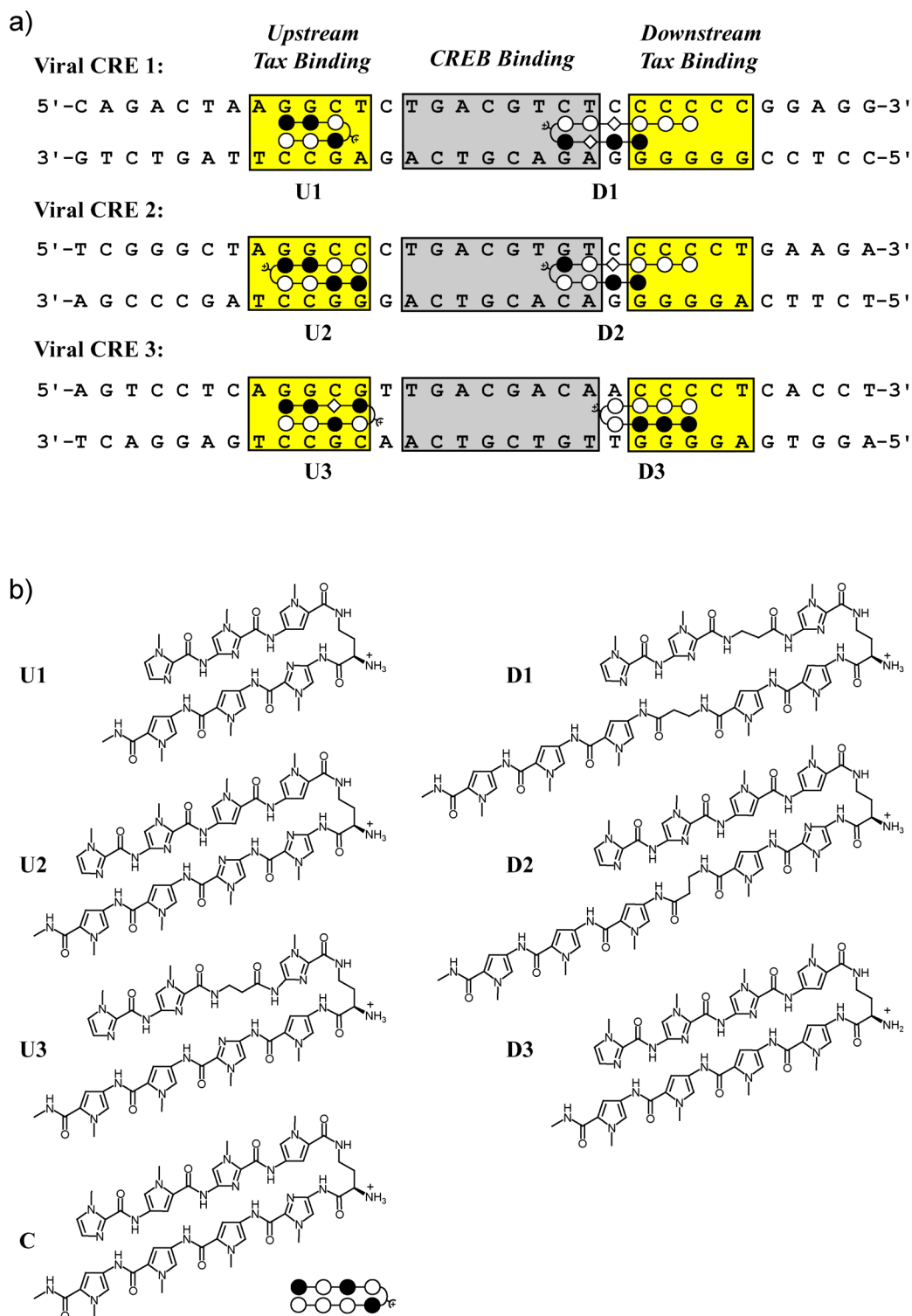


Figure A.2 (a) Nucleotide sequence of the viral CREs with the six target polyamides depicted in ball and stick models. Tax binding sequences are shown in yellow and CREB binding sites are shown in grey. (b) Structural representation of the six target polyamides and one control polyamide C.

U1 (upstream GC flank-viral CRE 1), **D1** (downstream GC flank-viral CRE 1), **U2** (upstream GC flank-viral CRE 1), **D2** (downstream GC flank-viral CRE 2), **U3** (upstream GC flank-viral CRE 3), and **D3** (downstream GC flank-viral CRE 3). Since polyamides bind exclusively in the minor groove of the DNA, these molecules were expected to compete with Tax for the binding to these sites on the viral promoter, as previously shown for the third proximal viral CRE.²⁴ A control polyamide (**C**), which recognizes the sequence 5'-WGWGCW-3', was synthesized and does not target any viral CRE region but rather the intervening sequences between the viral CREs.

Specific Polyamide Binding to Viral LTR Sequences

The polyamides were characterized by quantitative DNase I footprinting to assess specificity and affinity for their respective target sites (Figures A.3 and A.4). All polyamides bound their target sites with high affinity and specificity (Table A.1) with the exception of the hairpin **D1**, which was relatively non-specific and bound to a non-predicted site on the 3' side of CRE 2. As expected, the six-ring hairpin **U1** bound with the lowest affinity ($K_a = 4.7 \times 10^8$) while the larger nine-ring hairpin **D2** bound with the highest affinity ($K_a = 2.5 \times 10^{10}$). Notably, the control compound **C** bound at two sites located between each of the targeted CRE sequences with comparable affinity ($K_a = 1.7 \times 10^9$). Many of the designed polyamides bind to match or single base-pair mismatch sites within the promoter region potentially interacting with other essential transcription factors or altering the sensitive DNA architecture required for HTLV-1 transactivation.

Characterization of Polyamide Binding to Viral Promoter DNA

The electrophoretic mobility shift assay (EMSA) was used to assess polyamide

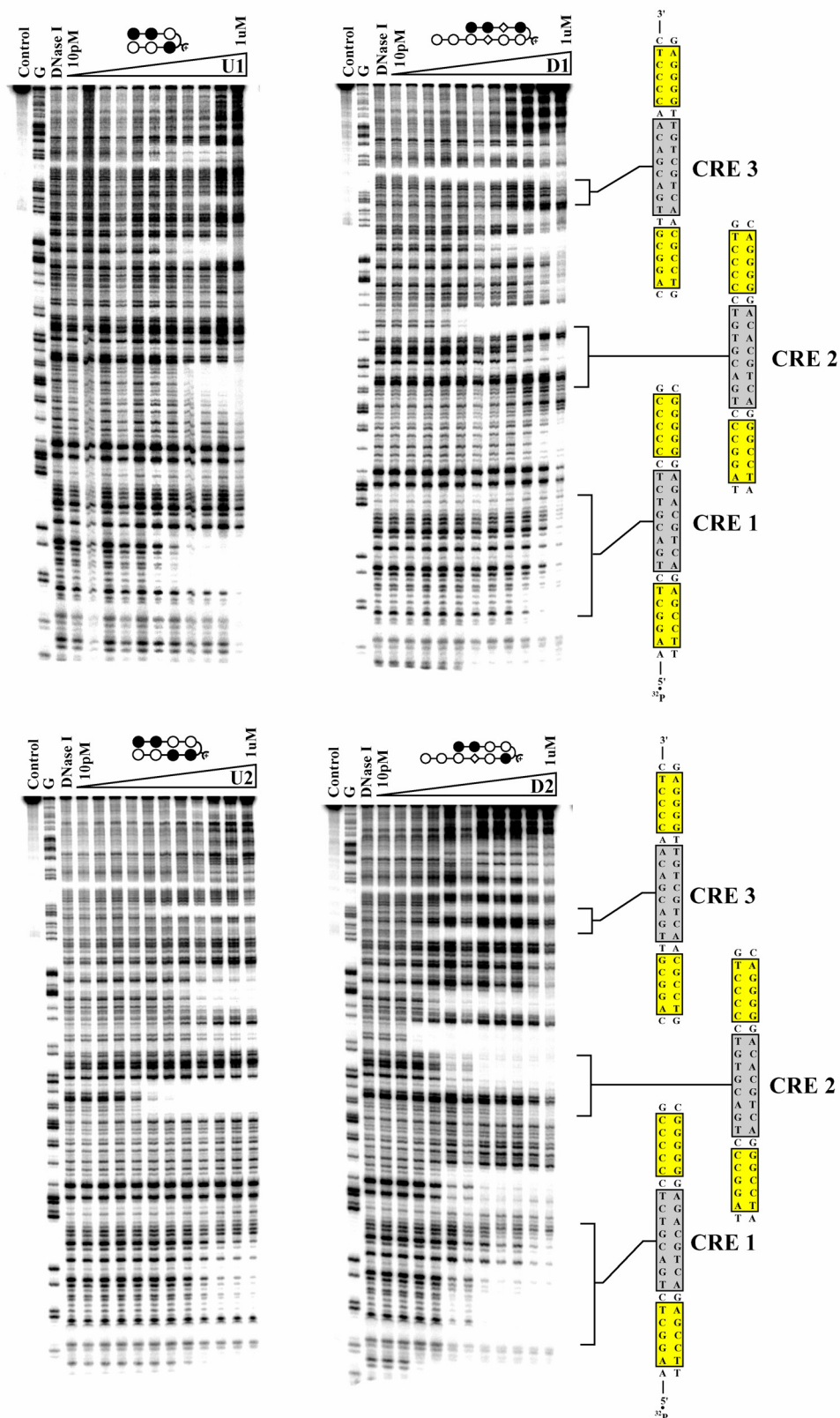


Figure A.3 Quantitative DNase I footprint titration experiments for viral CRE 1 and viral CRE 2 targeting compounds on a 5'-³²P-labeled DNA fragment containing the LTR region of the HTLV-1 promoter. All reactions contain 15 kcpm DNA fragment, 10mM Tris•HCl (pH 7.0), 10 mM KCl, 10mM MgCl₂, and 5 mM CaCl₂.

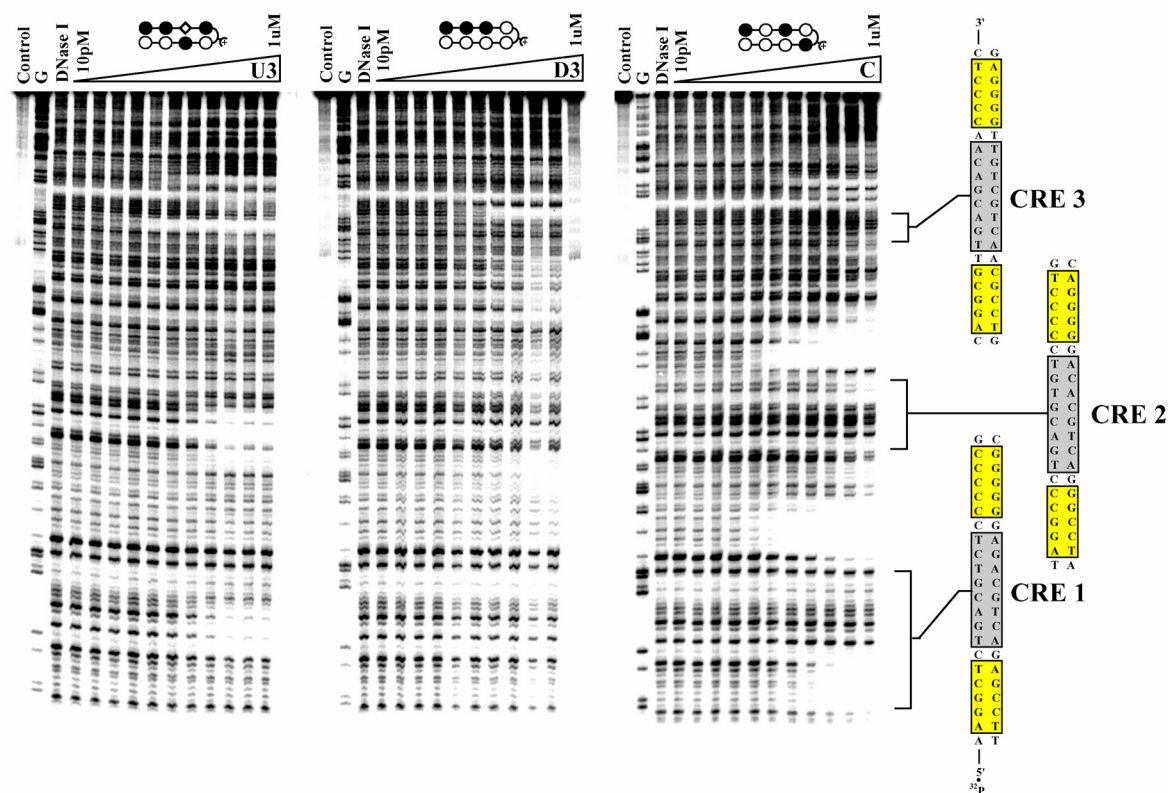


Figure A.4 Quantitative DNase I footprint titration experiments for viral CRE 3 targeting compounds and control C on a 5'-³²P-labeled DNA fragment containing the LTR region of the HTLV-1 promoter. All reactions contain 15 kcpm DNA fragment, 10mM Tris•HCl (pH 7.0), 10 mM KCl, 10mM MgCl₂, and 5 mM CaCl₂.

inhibition of Tax/CREB binding to their respective viral CRE DNA. In these experiments, individual viral CREs were incubated with a single targeted polyamide, recombinant Tax, CREB, and the KIX domain of CBP/p300. The KIX domain was used in these assays, as it enhances and stabilizes the Tax/CREB complex^{17,18,38}, thus enabling better visualization of potential binding inhibition by the polyamides. The polyamides were titrated into binding reactions, and their effect on Tax/CREB binding was quantitated and graphed (Figure A.5). The polyamides that inhibited Tax/CREB binding at 100 nM were U1 (Figure A.5b), D2 (Figure A.5c), and U3 (Figure A.5d). It is possible that targeting only one of the two GC-flanks was not sufficient to fully disrupt the

Tax/CREB/KIX complex from the DNA. The remaining polyamides, **D1**, **U2**, **D3**, and **C** inhibited Tax/CREB binding to the viral CREs only at their highest concentrations (1 μ M).

Effects of Polyamides on Tax-Mediated Transactivation

To characterize Tax function in the presence of polyamides, we tested polyamide effects on Tax-mediated transactivation via the HTLV-1 promoter. We performed *in vitro* transcription assays

using a plasmid carrying the natural HTLV-1 promoter cloned upstream of a 390 bp G-less cassette. A schematic representation of the HTLV-I promoter/transcription template is shown in Figure A.6a. Previous studies have shown that chromatin-based *in vitro* transcription systems are much more responsive to Tax.^{15,38,39} Therefore, we chose to analyze the effects of the polyamides in the context of chromatin, as this system better recapitulates the *in vivo* environment. Furthermore, polyamides have previously been shown to bind directly to nucleosomal DNA.⁴⁰ Chromatin assembly of the HTLV-1 G-less plasmid was performed using recombinant *Drosophila* assembly proteins Acf1/ISWI, Nap-1, and purified *Xenopus* core histones as previously described.^{15,28} These assembly proteins are sufficient for the ATP-dependent formation of evenly spaced nucleosomal arrays. Topological analysis assays determining the optimal ratio of histones to DNA


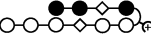
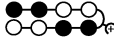
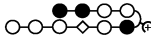
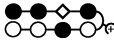
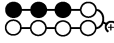
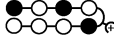
Polyamide		K_a (M^{-1})
U1		4.7×10^8
D1		**
U2		4.6×10^9
D2		2.5×10^{10}
U3		3.8×10^9
D3		8.5×10^8
C		1.7×10^9

Table A.1 Equilibrium association constants. Values reported are the mean values obtained from at least three independent DNase I footprint titration experiments. ** no specific binding to targeted site.

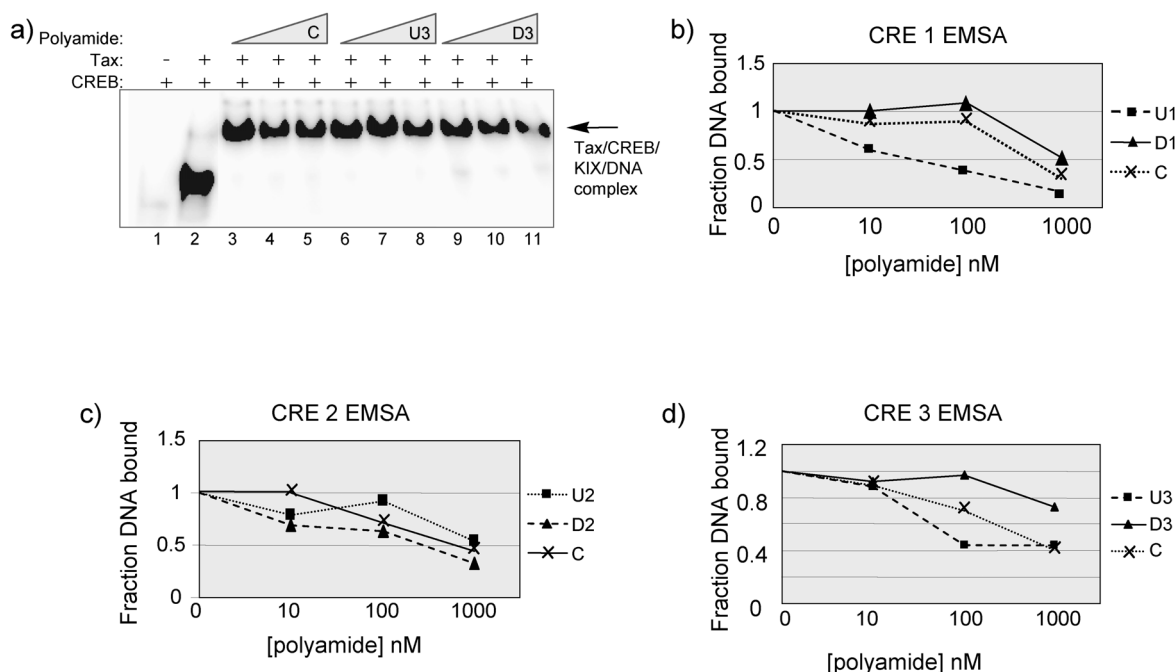


Figure A.5 Viral CRE targeted polyamides inhibit Tax/CREB binding to DNA. (A) Representative EMSA reveals Tax-polyamide competition for the viral CRE 3. Binding reactions for each EMSA were carried out using the relevant viral CRE probe, Tax/CREB/KIX, and polyamide. Reactions contained purified, recombinant Tax (375 fM), CREB (1 fM), GST-KIX aa 471-719 (200 fM), and increasing amounts (10, 100, 1000 nM) of the indicated polyamide. Protein-DNA complexes were resolved on a non-denaturing 5% polyacrylamide gel. The percent of protein bound as compared to free probe was quantified using ImageQuant, and the results were graphed and shown in panels B, C, and D.

were performed prior to the *in vitro* transcription studies (data not shown). We performed *in vitro* transcription assays on the HTLV-1 responsive chromatin template using nuclear extracts from CEM cells (a human T-lymphocyte cell-line) as a source of basal transcription factors, ATF/CREB proteins, and RNA polymerase II. All experiments were performed in the presence of acetyl CoA. Activator, coactivator, nuclear extract, and polyamides were all added following the assembly of nucleosomes on the transcription template. Addition of polyamides prior to preinitiation complex formation produced the same results as compared with adding the polyamides with the activator proteins during preinitiation complex formation (data not shown).

a) HTLV-I promoter/transcription template:

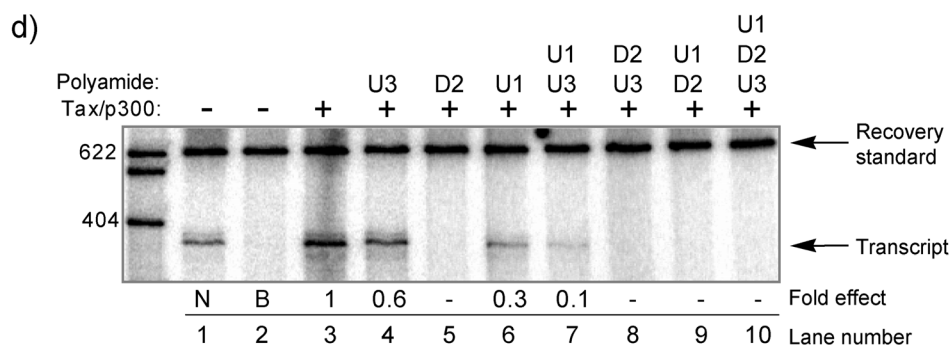
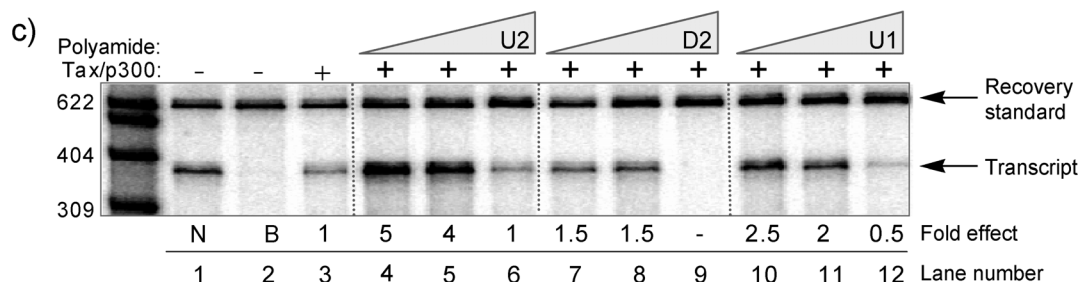
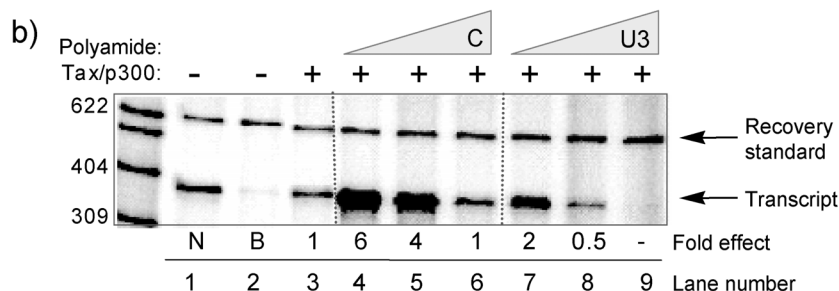
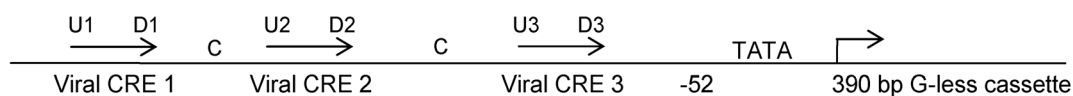


Figure A.6 Tax mediated transcription *in vitro* is inhibited by viral CRE targeted polyamides. (A) Schematic representation of the HTLV-I promoter/transcription template used in the *in vitro* transcription assays. (B) Transcriptional activation on the HTLV-I G-less, chromatin assembled template was analyzed in the presence of Tax (70 fM), p300 (30 fM), and 50 μ g of CEM nuclear extract. Polyamides were titrated at 10 nM, 100 nM, and 1 μ M, respectively. Molecular weight size markers, recovery standard, and the full-length G-less transcripts are indicated. The fold change, relative to the reaction containing Tax/p300 in the absence of polyamide, is indicated. The experiments shown in panels C and D were carried out as described in panel B except that 300 nM of each polyamide was used in the experiment shown in panel D.

Figure A.7b shows that the addition of Tax and p300 strongly stimulates transcription from the chromatin-assembled HTLV-I (compare lanes 2 and 3). Transcription on naked, or unassembled DNA, is also shown (Figure A.6b, lane 1). Polyamides were then titrated into the transcription reactions at concentrations of 10 nM, 100 nM, and 1000 nM, respectively. Of the five polyamides tested (**C**, **U3**, **U2**, **D2**, and **U1**), only **U3** inhibited Tax transactivation at 100 nM polyamide concentration (Figure A.6b, lane 8). **D2** and **U1** inhibited Tax transactivation at the highest concentrations of polyamide, whereas **U2** and **C** had no inhibitory effect at any of the concentrations tested (Figure A.6b and A.6c). Surprisingly, **U2** and **C** polyamides showed enhancement of Tax transactivation at the lower concentrations (Figure A.6b, lanes 4, 5; Figure A.6c, lanes 4, 5). Because this polyamide activation was not observed on naked DNA (data not shown), it may be due to nucleosome disruption, thus resulting in transcriptional activation.

We were next interested in determining whether multiple polyamides produced a more dramatic inhibitory effect on HTLV-I transcription. Figure A.6d shows the effect of combining polyamides in *in vitro* transcription assays at a concentration of 300 nM each. Transcriptional down-regulation was again observed in the presence of **U3**, **D2**, and **U1**, and the combination of **U3** and **U1** showed further inhibition than either polyamide alone (Figure A.6d, lanes 4, 6, 7). These data suggest that a combinatorial approach may be essential to achieve efficient inhibition of Tax function.

Effects of Polyamides on Basal and CREB Activation

To evaluate the specificity of the polyamides on Tax-dependent transcriptional activation, we were interested in testing the effect of the polyamides in CREB-mediated transcription from the HTLV-I promoter. Since the polyamides were designed to target

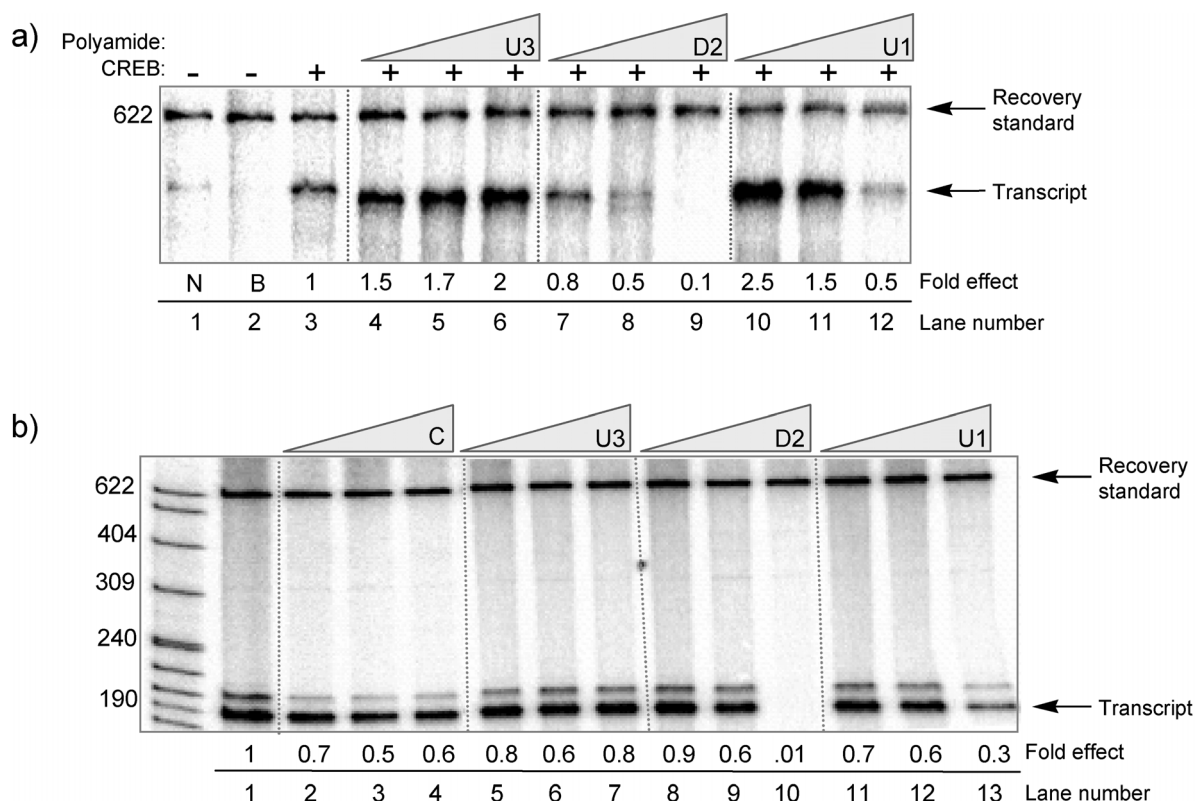


Figure A.7 Viral CRE-targeted polyamides inhibit CREB-mediated and basal transcription. (A) Transcriptional activation on HTLV-1 G-less chromatin assembled templates was analyzed in the presence of purified recombinant CREB (80 nM) and 50 μ g of CEM nuclear extract. Molecular weight size markers, recovery standard, and full-length G-less transcripts are indicated. The fold change was calculated relative to the reaction containing CREB in the absence of polyamide. Polyamides were titrated at 10 nM, 100 nM, and 1 μ M, respectively. (B) Transcription from the HTLV-1 G-less DNA templates, in the absence of chromatin, was analyzed in the presence of 50 μ g of CEM extract. Molecular weight size markers, recovery standard, and full-length G-less transcripts are indicated. The fold activation indicated was calculated relative to transcription in the absence of polyamide. Polyamides were titrated at 10 nM, 100 nM, and 1 μ M, respectively.

the GC-rich sequences flanking the CRE, and since they bind in the minor groove, these molecules were predicted to have no effect on CREB binding. We added recombinant CREB to chromatin-assembled DNA and titrated the polyamides into the reaction. Interestingly, we found polyamides **D2** and **U1** strongly inhibited CREB-mediated transcription (Figure A.7a lanes 8, 9, and 12). Only one of the polyamides (**U3**) had no effect on CREB-dependent transcription from the HTLV-1 promoter. These data suggest

that the transcriptional inhibitor effects of **D2** and **U1** are not Tax-specific. Furthermore, they provide support for the specificity of polyamide **U3** in Tax-mediated transcriptional activation.

To further address the specificity of the polyamides, we used an HTLV-1 promoter construct carrying only the first 52 bases upstream of the core HTLV-1 promoter and lacking all three viral CREs (see Figure A.2a). This promoter is not Tax responsive, allowing us to monitor basal transcription from the HTLV-1 core promoter. These transcription assays were performed in the absence of chromatin, as the core HTLV-1 promoter is not responsive to transcriptional activators. Consistent with the above results, we found that polyamides **D2** and **U1** down-regulated basal HTLV-1 transcription, whereas polyamide **U3** had no effect (Figure A.2a). This observation further identifies polyamide **U3** as the only polyamide that specifically inhibits Tax-mediated transcription. All of the others appear to inhibit non-specifically or have no effect.

Polyamides Enter the Nucleus of Living HTLV-1 Infected T-cells

We were also interested in correlating our *in vitro* polyamide studies with viral expression in HTLV-1 infected cells. It has been shown that polyamides enter the nucleus of certain types of T-cells.⁴¹ Therefore, we tested the ability of our HTLV-1 infected cell lines to take up fluorescently labeled polyamides into their nuclei. A sequence specific hairpin polyamide-Bodipy conjugate (Figure A.8a) was incubated with two HTLV-1 infected cultured T-cell lines, SLB-1 (Figure A.8b) and MT2 (Figure A.8c). Figure 5 shows that both cell lines exhibited nuclear uptake at all concentrations tested. Cells were also incubated with a mitochondrial staining dye to confirm cell viability. Figure A.8c

(right panel) shows that the polyamides do not cause cell death or apoptosis. These experiments indicate that the polyamides enter the nucleus of living HTLV-1 infected T-cells and therefore are available for potential effects on gene expression.

Polyamides Inhibit Viral Replication in Cell Culture

With the knowledge that polyamides efficiently localize to the nucleus of HTLV-1 infected cells, we began studying the effect of polyamides on HTLV-1 expression in living cells. If certain polyamides blocked Tax/CREB binding to the HTLV-1 promoter, perhaps they also downregulate viral replication, inhibiting virion production. To test this, we incubated both SLB-1 and MT2 cells with the small library of polyamides and measured virion levels by ELISA monitoring p19. Both of these HTLV-1 infected cell lines carry

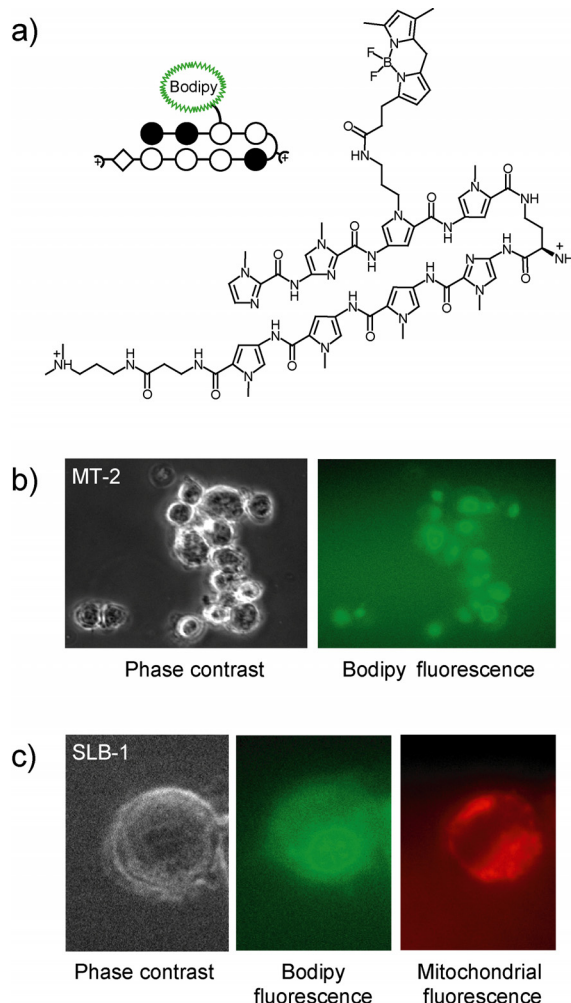


Figure A.8 Polyamides enter the nucleus of living HTLV-1 infected T-cells. Cells were incubated with a bodipy conjugated polyamide (5 μ M) for 20 hr. under normal growth conditions for these cell lines. The cells were visualized by confocal microscopy. (A) Structure of Polyamide-Bodipy Conjugate used for cellular uptake study. (B) MT-2 cells. Left panel, phase contrast image; right panel, bodipy fluorescence. (C) Oil of immersion image of an SLB-1 cell. Left panel, phase contrast image; middle panel, bodipy fluorescence; right panel, mitochondrial fluorescence following incubation with TMRM, a dye specific for active mitochondria.

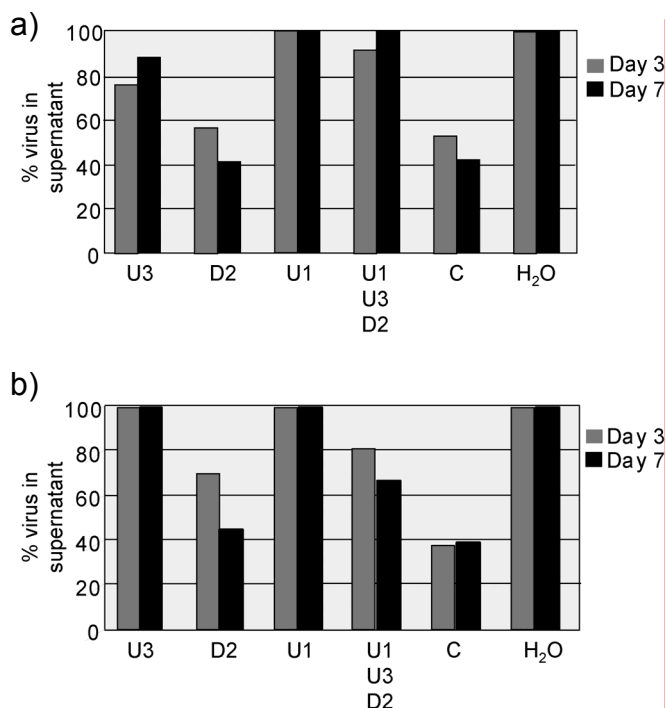


Figure A.9 Polyamides inhibit virion production in living HTLV-1 infected cells. Polyamides were incubated with living cells to a final concentration of 7.5 μ M for 3 or 7 days as indicated. Culture supernatant was harvested and analyzed by ELISA testing for viral p19 antigen. (A) SLB-1 cells. (B) MT-2 cells.

multiple proviruses and express large amounts of Tax.⁴² We used a polyamide concentration of 7.5 μ M, as this is below the level of polyamide toxicity.⁴³ We tested the polyamides that were previously shown to be successful at inhibition of Tax/CREB binding and HTLV-1 transcription *in vitro*. Polyamides **D2** and **C** produced the most dramatic effects on virion production in both cell types examined (Figures A.9a, A.9b). Remarkably, neither of these polyamides were shown to have

specific effects on Tax-activated transcription *in vitro*. In contrast, polyamide **U3**, which had the most dramatic Tax-specific effects *in vitro*, had essentially no effect in living cells.

Discussion

Sequence specific DNA binding polyamides have the potential to be effective molecules for inhibiting protein binding to DNA.²³ Their specificities and affinities approach those of naturally occurring DNA binding proteins, thus making them good candidates for a chemical approach to modulation of transcription. They have been shown to effectively inhibit the binding to factors of promoters such as HIV⁴⁴, TBP

binding⁴⁵, Polymerase II⁴⁶, TFIIA⁴⁷, Human Papilloma virus E2⁴⁸, NF- κ B⁴⁹, and MMTV.⁵⁰ Although *in vitro* studies displaying their effectiveness at inhibiting transcription factor binding are positive and promising, their function *in vivo* is not fully understood.

A previous study from our laboratory showed that two minor groove-binding polyamides were effective at inhibiting HTLV-1 Tax protein from binding to its promoter DNA sequence.²⁴ In the present study, we designed and synthesized six different polyamides targeted to GC-rich viral CRE sequences in the HTLV-1 promoter. These studies were carried out with the ultimate goal of disrupting Tax binding and transcriptional activation *in vitro* and inhibiting viral replication *in vivo*. We demonstrated that three of the six polyamides disrupt Tax/CREB binding, whereas only one (**U3**) inhibited Tax-specific transcription. The others either had no effect or were non-specific in their effects. Interestingly, two polyamides (**D2** and **C**) were effective at inhibiting viral replication in cell culture; neither of these polyamides were shown to be Tax specific *in vitro*. The control polyamide **C**, which was designed to bind the HTLV-1 promoter but *not* to bind the viral CREs, turned out to be one of the most potent inhibitors of HTLV-1 in living cells. Analysis of polyamide **C** binding to the HTLV-1 promoter by DNase footprinting shows that this polyamide binds between the viral CREs. Although Tax does not bind in this region, it is possible that another factor may interact in this region that could play a prominent role in transcription. Multiple cellular factors have previously been shown to bind between the second and third viral CREs.^{51,52} Alternatively, it may be that polyamide **C** exerts its effect as an architectural cofactor, perhaps stiffening the DNA in regions that should be bent in a higher order protein-DNA

complex.

Since we do not fully understand the biology of HTLV-1 transcription, we may not be able to accurately predict the effect of rationally designed DNA binding ligands on viral regulation. Perhaps a better way to search for polyamides that successfully inhibit HTLV-1 replication will be to screen a library of molecules for their effectiveness at specifically reducing viral replication. Following the initial screening and identification of “active” polyamides, an investigation into the molecular mechanisms of their mode of inhibition may lead to interesting new discoveries about the biology of HTLV-1 transcription. This approach may also lead to the identification of effective polyamides that may be useful as pre-clinical lead structures for anti-HTLV-1 therapeutics.

Experimental

Purification of Recombinant Proteins

Tax was expressed from the pTaxH₆ expression plasmid and purified as previously described;^{17,25} His₆-tagged p300 was expressed from recombinant baculovirus in Sf9 cells and purified as previously described;²⁶ and full-length recombinant CREB was expressed and purified as previously described.^{17,27} Expression and purification of GST-KIX (a.a. 471-719) has been previously described.¹⁷ Purified proteins were dialyzed against TM buffer (50mM Tris-HCL [pH 7.9], 100 mM KCl, 12.5 mM MgCl₂, 1 mM EDTA [pH 8.0], 1 mM dithiothreitol, 20% [v/v] glycerol) and stored at −70°C. Drosophila NAP-1 (dNAP-1) (His₆ tagged) was expressed from recombinant baculovirus in Sf9 cells and purified Ni²⁺-agarose batch binding and elution followed by Source 15Q column chromatography.^{28,29} FLAG-tagged ISWI and Acfl were co-expressed from

baculovirus in Sf9 cells, and the complex was purified by anti-FLAG affinity batch binding and eluted as previously described.³⁰ The four core histones were individually expressed in *E. coli* and purified to homogeneity as previously described.³¹ Histone octamers were prepared by denaturation, then renaturation with high levels of salt followed by purification of the octamers by gel filtration and ion exchange chromatography. CEM and SLB-1 nuclear extract were purified as previously described.³²

***In vitro* Transcription**

The HTLV-1 promoter G-less cassette template and –52 G-less cassette template have been previously described.³³ The basal HTLV-1 promoter construct carried the –52 to +1 HTLV-1 promoter sequences followed by a 190 bp G-free cassette.³³ The supercoiled plasmids were assembled into chromatin using dNAP-1, ACF, and *Xenopus* histones at a 0.57:1.0 histone:DNA ratio.²⁸ Following assembly, preinitiation complexes were formed on the equivalent of 150 ng of the plasmid DNA in the absence or presence of Tax (100 ng), p300 (150 ng), and polyamide. All reactions contained 100 μ M acetyl CoA. CEM (a human T lymphocyte cell line) nuclear extract (50 μ g) was added immediately following the addition of the activator and coactivator. Following a 60 minute preincubation reaction, RNA synthesis was initiated by the addition of 250 μ M ATP, GTP and CTP, and 12 μ M UTP plus 0.8 μ M ³²P-alpha-UTP (3000 Ci/mmol). Transcription reactions were processed and analyzed as previously described.²⁴ Molecular weight markers (radiolabeled *Hpa* II digested pBR322) were used to estimate the size of the RNA products.

Electrophoretic Mobility Shift Assay

Protein-DNA complexes were resolved by non-denaturing polyacrylamide gel electrophoresis. EMSAs were performed by incubation of the indicated amount of purified proteins and polyamides. The appropriate ^{32}P -end-labeled DNA (4 fmol) probe was used in a 20- μl reaction mixture. The polyamides were incubated with the DNA for 20 min at room temperature before protein addition. The proteins were added, the reaction tube was incubated on ice for 30 min, and its contents were analyzed on 5% nondenaturing polyacrylamide gels (acrylamide/N,N'-methylenebisacrylamide, 36.5:1 [wt/wt]) in buffer containing 0.04 M Tris-HCl, 0.306 M glycine (pH 8.5), and 0.1% (v/v) Nonidet P-40. The gels were visualized with a PhosphorImager.

ELISA

Kits were purchased from Zeptometrix Corporation, and experiments were carried out according to the manufacturer's protocol. HTLV-1-transformed T-cell lines (SLB-1 and MT2) were incubated with 2.5 μM of the indicated polyamides. Cells were counted daily, kept in log-phase growth, and assayed for viability with trypan blue. Polyamides at these concentrations did not produce any adverse effects on cell growth. At days 3 and 7 culture supernatants were harvested and diluted 1:1000 for testing of viral p19 antigen.

Cell Culture

HTLV-1 transformed T-cells (SLB-1 and MT-2) were cultured in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin/streptomycin.

Polyamide Synthesis

Polyamides were synthesized using solid-phase methods.³⁴ Polyamides were resuspended in H₂O and the concentration was assayed by spectrophotometer. Chemical structures of each of the polyamides are as follows: ImImPy-(*R*)^{H₂N} γ -ImPyPyCONHMe (**U1**), ImIm- β -Im-(*R*)^{H₂N} γ -PyPy- β -PyPyPyCONHMe (**D1**), ImImPyPy-(*R*)^{H₂N} γ -ImImPyPyCONHMe (**U2**), ImImPyPy-(*R*)^{H₂N} γ -ImPy- β -PyPyPyCONHMe (**D2**), ImIm- β -Im-(*R*)^{H₂N} γ -PyImPyPyCONHMe (**U3**), ImImImPy-(*R*)^{H₂N} γ -PyPyPyPyCONHMe (**D3**), and ImPyImPy-(*R*)^{H₂N} γ -ImPyPyPyCONHMe (**C**), where Im = imidazole, Py = pyrrole, β = β -alanine, and (*R*)^{H₂N} γ = (*R*)-diaminobutyric acid.

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